Effect of Cold Stress on Broilers Selected for Resistance or Susceptibility to Ascites Syndrome[1]

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ABSTRACT Genetic selection for an ascites-resistant line of broilers is seen as a permanent solution to the ascites problem. Ascites-resistant and ascites-susceptible lines have been developed using sire family selection based on mortality data taken from siblings reared in a hypobaric chamber (simulated 2,900 m above sea level). The relaxed line is representative of the original commercial pureline stock randomly mated with no artificial selection pressure. The objectives of this study were to evaluate the differences between the lines when reared in floor pens and subjected to an ascites-inducing cold stress. Seven hundred eighty three straight run broilers were reared in floor pens at local elevation (390 m above sea level). Feed and water were available ad libitum. Birds were brooded at 32 C during the first week. The second week birds were maintained at 30°C. Cold stress was applied for the remaining 4 wk at 14°C. Mortalities were necropsied daily to determine cause of death. Birds and feed were weighed weekly. At 6 wk, five birds per pen were bled, and half the survivors in each pen (8 to 15 birds) were killed, necropsied, and scored for ascites. Blood gases, clinical chemistries, and blood cell counts were taken. Liver, spleen, split heart, and lung weights were recorded. Body weights were not different among the resistant, susceptible, and relaxed lines ($P < 0.05$). Feed conversion was better in the resistant line when compared to the susceptible and relaxed lines ($P < 0.05$). Ascites incidence, as measured by mortality and lesion score at necropsy, was higher in the susceptible and relaxed lines when compared to the resistant line; 18.8, 12.7, and 1.6% respectively ($P < 0.001$). Susceptible and relaxed lines showed more right ventricular hypertrophy when compared with the resistant line ($P < 0.05$). The results show that under severe cold stress at local altitude (390 m above sea level), the ascites-resistant line was growing as rapidly as the other lines and was as resistant to ascites as it had been under hypobaric conditions.

(*Key words*: ascites, genetic selection, body weight, feed conversion, ascites mortality)

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INTRODUCTION

Ascites is a metabolic disease of fast-growing meat-type birds. The incidence of ascites is influenced by both environmental and genetic factors. There are several clinical signs associated with ascites syndrome in broiler chickens: pulmonary hypertension, right ventricular hypertrophy, central and portal venous congestion, hepatic damage, and transduction of fluid into the abdominal cavity (Riddell, 1991; Yersin et al., 1992; Julian, 1993; Wideman et al., 1995). During the development of ascites syndrome, birds exhibit classic hematological changes. Hematocrit, hemoglobin, and red blood cell counts (RBC) all increase dramatically (Hall and Machicao, 1968; Cueva et al., 1974; Maxwell et al., 1986, 1987; Yersin et al., 1992). In hypoxic situations, it has been postulated that lowered oxygen tensions reduce capillary blood flow, and, in combination with the polycythemia, ascites development is hastened (Olander et al., 1967). Right ventricle to total ventricle (RV/TV) ratio [wt (g) / wt (g)], hemoglobin, hematocrit, blood gases, and specific clinical chemistries can be used to determine the ascites status of a bird before gross lesions are apparent. An RV/TV ratio of > 0.27 is considered an accurate measure of the onset of ascites (Huchzermeyer and DeRuyck, 1986; Peacock et al., 1988).

Genetic selection based on rapid growth rates, better feed conversion, and heavier BW of broilers has led to a predisposition to ascites in broiler populations. Genetic selection of an ascites-resistant line of broilers could be a permanent solution to the ascites problem. It seems reasonable that an ascites-resistant line could be selected,

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Abbreviation Key: RBC = red blood cells; RV = right ventricle; TV = total ventricle.

as Lubritz et al. (1995) found moderate to high heritability for ascites and moderate heritability for the ratio of right ventricle to total heart weight in three lines of broiler breeders.

Experimental induction of ascites can be accomplished in several ways, including cold stress, hypobaric exposure, and surgical occlusion. Each experimental model induces ascites through a slightly different mechanism. The hypobaric chamber induces ascites by operating under a partial vacuum, which lowers the oxygen availability for the birds. A hypobaric chamber model that simulates an altitude of 2,900 m (9,500 ft) above sea level (Hughes et al., 1995; Balog et al., 2000a,b; Pavlidis et al., 2002) has been successful at inducing ascites in broilers. Cold stress appears to increase the metabolic demand for oxygen, whereas surgical occlusion physically decreases the amount of oxygen or oxygenated blood available to the bird.

The objective of the research reported here was to determine the physiological and anatomical differences between divergent lines of birds selected for ascites under hypobaric conditions as compared to an unselected parent (relaxed) line when reared in floor pens and subjected to cold stress.

MATERIALS AND METHODS

Line Selection

Birds used in this study were taken from the fifth generation of an ongoing ascites selection study (Anthony et al., 2001; Pavlidis et al., 2002). Ascites-resistant and susceptible broiler lines were derived from a commercial elite line that had undergone a single generation of relaxed selection prior to initiation of ascites selection. The relaxed line is maintained as a randomly bred population with no particular selection pressure being applied and continues to represent the outstanding growth found in the commercial broiler of today.

For the divergent lines, sire-family selection was applied. Each generation, two hatches of progeny derived from 24 sires mated to three hens per sire were reared for 6 wk under simulated high altitude conditions in a hypobaric chamber (Hughes et al., 1995; Balog et al., 2000a,b; Pavlidis et al., 2002). Body weight was measured at 3 and 6 wk. All mortalities were posted to determine if ascites syndrome was present. All survivors were necropsied and scored for ascites at 6 wk. The mortality data generated from the altitude challenges were used to select the most susceptible and resistant sire families to reproduce the lines. For each line, male breeders were selected from the top six sire families and hens were selected from no more than the top 10 sire families. Blood samples were collected from all breeders selected each generation and frozen for future analysis.

After five generations of selection, under hypobaric conditions the ascites-resistant line exhibits no more than 30% mortality, the ascites-susceptible line exhibits no less than 75% ascites mortality, and the relaxed line exhibits 65% ascites mortality.

Cold Stress Trial

Broilers from the ascites-resistant, ascites-susceptible, and relaxed lines were placed in floor pens at day of hatch. Each line was randomly assigned to eight replicate floor pens (at least 30 chicks/pen). Each pen (2.13×1.68 m) was equipped with a bell waterer and tube feeder, with feed and water available ad libitum. The mash starter and the pelleted grower were corn-soybean diets that met or exceeded the National Research Council (NRC, 1994) requirements. Birds and feed were weighed weekly. Mortalities were weighed and necropsied to determine if ascites was present. A lighting cycle of 23L:1D was implemented. Chicks were warm-room brooded at 32°C during the first week and 30°C for the second week. Starting at d 14 and continuing through wk 6, birds were cold-stressed at an average room temp of 14°C. Room temperature was controlled through ventilation and fluctuated slightly.

On d 41, blood samples were obtained by cardiac puncture from five randomly selected birds per pen. Total RBC, differential white blood cell counts, and hematology values were determined using a Cell-Dyn 3500 blood analysis system[3] that was standardized for analysis of chicken blood. Serum was analyzed with a Corning Express Plus clinical chemistry analyzer, and reagents and procedures were standardized for use with the analyzer.[4] Blood gases were analyzed using a Radiometer ABL-70 pH, blood gas, and electrolyte analyzer[5] unit on site of necropsy.

On d 42, half the remaining birds in each pen (8 to 15 per pen) were killed by cervical dislocation, weighed, necropsied, and scored for ascites. Ascites scores were assigned as follows: 0 (normal, no sign of heart or liver involvement, no fluid in abdominal cavity), 1 (mild heart enlargement, with flaccidity and possible hydropericardium), 2 (distinct, severe heart enlargement with minimal pericardial or abdominal fluid accumulation), and 3 (most severe ascites, characteristic right ventricular hypertrophy, copious abdominal fluid with or without fibrin clots, congested and edematous lungs and liver). Hearts were split, and right ventricle (RV) and total ventricle (TV) weights were obtained. Liver, lung, and spleen weights were also obtained.

All percentage data were subjected to arc sine transformation (Steel and Torrie, 1960). The data were analyzed by general linear model procedure of SAS software (SAS Institute, 1988). When necessary, mean separation was accomplished using Duncan's multiple-range test (Duncan, 1955). A probability value of less than 0.05 was considered significant, unless otherwise noted. The experimental design was a completely randomized design. This experimental protocol was approved by the University

[3]Abbott Diagnostics, Abbott Park, IL.
[4]Ciba-Corning Diagnostic Corp., Medfield, MA.
[5]Radiometer America, Inc., Westlake, OH.

TABLE 1. Live BW at 1, 2, 3, 4, 5, and 6 wk of age in broilers from a relaxed line and in lines selected for resistance or susceptibility to ascites while subjected to cold stress[1]

Week	Resistant	Susceptible	Relaxed
	(g)		
1	118 ± 1[a]	111 ± 1[b]	120 ± 1[a]
2	313 ± 4[b]	323 ± 3[ab]	332 ± 4[a]
3	621 ± 8[b]	645 ± 7[a]	650 ± 7[a]
4	1,110 ± 13[b]	1,118 ± 11[b]	1,155 ± 11[a]
5	1,514 ± 17	1,480 ± 17	1,535 ± 17
6	1,958 ± 22	1,942 ± 23	2,006 ± 23

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Means ± SE.

TABLE 3. Cumulative ascites mortality at 1, 2, 3, 4, 5, and 6 wk of age in broilers from a relaxed line and in lines selected for resistance or susceptibility to ascites while subjected to cold stress[1]

Week	Resistant	Susceptible	Relaxed
	(%)		
1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	0.4 ± 0.4	0.7 ± 0.4	0.4 ± 0.4
4	0.8 ± 0.5	3.4 ± 1.4	2.9 ± 1.3
5	0.8 ± 0.5[c]	13.1 ± 2.5[a]	6.2 ± 1.3[b]
6	1.2 ± 0.6[b]	15.8 ± 2.9[a]	10.7 ± 2.4[a]

[a–c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Means ± SE.

of Arkansas Institutional Animal Care and Use Committee and complied with university and federal guidelines for research involving animal subjects.

RESULTS AND DISCUSSION

While under cold stress in floor pens, there were no significant differences in final live BW for any of the lines (Table 1). There were some significant differences in live BW during wk 1 to 4, indicating perhaps that selection for ascites resistance or susceptibility altered the birds' growth curve (Table 1). The unselected relaxed line was consistently heaviest, whereas the resistant and susceptible lines varied from week to week (Table 1). Most importantly however, during wk 5 and 6 there were no differences in live BW among the lines (Table 1). Resistant birds gained weight just as rapidly as the susceptible birds. Past research and common commercial practices have shown that decreasing growth rate decreases ascites incidence (Arce et al., 1992; Robinson et al., 1992; Yu and Robinson, 1992; Acar et al., 1995; Cooper et al., 1998; Balog et al., 2000a), and so it was suspected that intense selection pressure on ascites incidence might have the undesirable effect of reducing BW. This result was not apparent in this floor pen trial. Intense production trials need to be conducted to see if the lines respond in a similar manner when there is no cold stress applied.

Feed efficiency was unaffected by line for the first 3 wk of the trial (Table 2). However, at wk 5 the relaxed line was significantly less efficient when compared with the selected lines (Table 2). Ultimately, at 6 wk the relaxed and the susceptible lines were significantly less efficient at feed conversion when compared with the resistant line. It is unusual, but advantageous, to have an intensely selected line that exhibits a better feed conversion.

Table 3 and Figure 1 indicate how the lines responded in terms of ascites mortality and ascites incidence. There was no ascites mortality during the first 2 wk, before the cold stress was applied (Table 3). During wk 3 and 4, there was some ascites mortality but there were no differences among the lines (Table 3). During the final 2 wk, the three different lines responded as they had in the hypobaric chamber (Anthony et al., 2001). The ascites-susceptible line exhibited the most ascites mortality (15.8 %), when compared with the ascites-resistant line (1.2 %) (Table 3). The relaxed line had intermediate ascites mortality (10.7 %). The effect of line differences on ascites mortality is also evident in the percentage of total mortality that is accounted for by ascites. At 6 wk, 76% of the total mortal-

TABLE 2. Feed efficiency at 1, 2, 3, 4, 5, and 6 wk of age in broilers from a relaxed line and in lines selected for resistance or susceptibility to ascites while subjected to cold stress[1]

Week	Resistant	Susceptible	Relaxed
	(g:g)		
1	1.59 ± 0.05	1.49 ± 0.04	1.46 ± 0.03
2	1.17 ± 0.02	1.18 ± 0.02	1.20 ± 0.03
3	1.32 ± 0.02	1.34 ± 0.02	1.39 ± 0.04
4	1.53 ± 0.02[ab]	1.49 ± 0.02[b]	1.58 ± 0.03[a]
5	1.67 ± 0.02[b]	1.67 ± 0.02[b]	1.77 ± 0.03[a]
6	1.76 ± 0.02[b]	1.90 ± 0.04[a]	1.91 ± 0.03[a]

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Means ± SE.

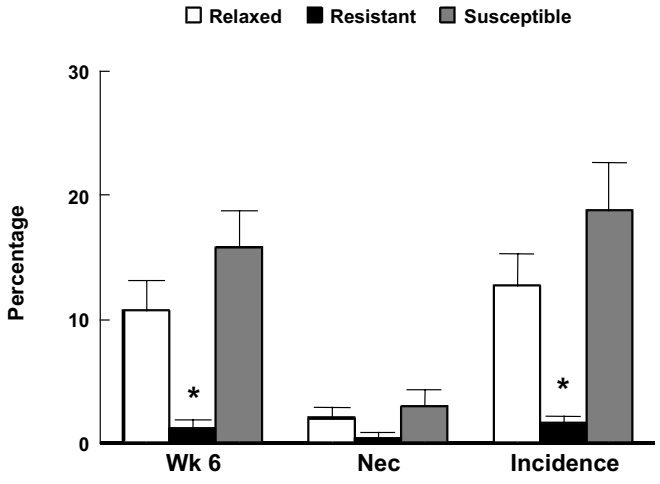

FIGURE 1. Cumulative ascites mortality through wk 6 as a percentage of birds placed, percentage ascites observed at necropsy (Nec) on d 42, and percentage overall ascites incidence of the three lines of broilers [relaxed (n = 242), resistant (n = 242), and susceptible (n = 297)]. Results represent means, and lines are SE. *Means within a group differ significantly ($P < 0.05$).

TABLE 4. Organ weights at 6 wk of broilers from a relaxed line and in lines selected for resistance or susceptibility to ascites while subjected to cold stress[1]

Parameter	Resistant	Susceptible	Relaxed
Relative heart [(heart g/BW g) × 100]	0.38 ± 0.00[b]	0.41 ± 0.01[a]	0.40 ± 0.01[a]
Right ventricle/total ventricle (g/g)	0.200 ± 0.003[c]	0.252 ± 0.007[a]	0.223 ± 0.005[b]
Relative liver [(liver g/BW g) × 100]	2.68 ± 0.04[b]	2.65 ± 0.04[b]	2.92 ± 0.05[a]
Relative spleen [(spleen g/BW g) × 100]	0.16 ± 0.00[b]	0.17 ± 0.00[ab]	0.18 ± 0.01[a]

[a–c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Means ± SE.

ity in the susceptible line was due to ascites, whereas only 32% of the total mortality in the resistant line was due to ascites. The relaxed line was intermediate with 62% of total mortality being caused by ascites. Figure 1 summarizes the ascites incidence observed in this trial. We defined the incidence of ascites for this trial to be the percentage of birds that died of ascites during the 6-wk period combined with the percentage of birds exhibiting pathological signs of ascites at the wk 6 necropsy. Figure 1 shows that for all three measures of the incidence of ascites, the line selected to be ascites resistant under hypobaric conditions had the lowest ascites observed compared with the ascites-susceptible and the unselected relaxed lines, when exposed to cold stress. Although the hypobaric model and the cold stress model of ascites induction use different methods to induce hypoxia, the lines that were selected for resistance to hypobaric or low-oxygen stress were also most resistant to the cold or metabolic demand stress. In a hypobaric chamber, the magnitude of the ascites incidence response was greater (86% susceptible, 66% relaxed, and 22% resistant) (Anthony et al., 2001) as compared with this cold-stress trial (18.8% susceptible, 12.7% relaxed, and 1.6% resistant), but the shape of response graph remained the same.

Before a bird exhibits gross ascites syndrome lesions, there are commonly hematological and anatomical changes that can be detected (Maxwell et al., 1986, 1987). In this trial, there were no significant line differences for any of the hematological parameters investigated (data not shown). Although RBC, hematocrit, and hemoglobin levels showed a consistent pattern of hierarchy (susceptible highest, relaxed intermediate and resistant lowest), none was significant. This result was not surprising as a very small percentage of birds, in any of the lines, exhibited ascites or pre-ascitic changes at necropsy (Figure 1).

It is also known that various organ changes and an increase in the RV/TV ratio indicate the onset of pulmonary hypertension and ascites syndrome (Burton et al., 1968; Cueva et al., 1974; Huchzermeyer and DeRuyck, 1986; Hernandez, 1987). Table 4 shows the effects of cold stress on various organ weights, obtained at necropsy. Relative heart weight was significantly higher in the susceptible and the relaxed lines, when compared with the resistant line (Table 4). There were also significant differences in RV/TV ratios at 6 wk (Table 4). It is generally accepted that an RV/TV index greater than 0.27 to 0.30 is indicative of right ventricular hypertrophy, pulmonary hypertension, and ultimately, ascites syndrome (Cueva et al., 1974; Hernandez, 1987; Huchzermeyer et al., 1988; Wideman et al., 1998). Although none of the lines exceeded this 0.27 limit, the susceptible line and the relaxed lines exhibited significantly more right ventricular hypertrophy than the resistant line. It is unclear whether or not these birds would have eventually developed full ascites, but early heart changes seem to indicate that they would.

Although liver and spleen changes do occur with ascites syndrome, the type of direction of the change varies with the course of the disease. In this trial, the relaxed line had significantly heavier relative liver and spleen weights when compared to the resistant line (Table 4). This finding seems to be a further indication of more pre-ascitic birds in this line.

There were no significant line differences in blood oxygen, pH, serum calcium, total protein, albumin, creatine kinase, glucose, or iron (data not shown). There were significant line differences in blood carbon dioxide, and serum phosphorus, uric acid, alkaline phosphatase, and triglycerides (Table 5). Blood carbon dioxide was significantly higher in the susceptible and relaxed lines when compared with resistant birds, which indicates less hypoxia in the resistant line. The relaxed and the susceptible lines also exhibited significantly decreased serum phosphorus and alkaline phosphatase levels, when compared to the resistant line. This reduction in phosphorus and

TABLE 5. Blood gas and clinical chemistries (d 41) of broilers from a relaxed line and in lines selected for resistance or susceptibility to ascites while subjected to cold stress[1]

Parameter	Resistant	Susceptible	Relaxed
Blood CO_2 partial pressure (mm of Hg)	48.6 ± 1.0[b]	53.1 ± 1.0[a]	50.4 ± 1.0[ab]
Serum phosphorus (mg/dL)	7.64 ± 0.17[a]	7.14 ± 0.14[b]	7.07 ± 0.15[b]
Serum uric acid (mg/dL)	8.32 ± 0.32[b]	9.00 ± 0.35[b]	10.16 ± 0.31[a]
Serum alkaline phosphatase (U/L)	476 ± 59[a]	318 ± 51[b]	301 ± 32[b]
Serum triglycerides (mg/dL)	125.2 ± 6.5[b]	112.6 ± 6.5[b]	148.2 ± 8.7[a]

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Means ± SE.

alkaline phosphatase is commonly seen during ascites development (J. Balog, unpublished data). Conversely, increases in uric acid are frequently seen as ascites develops (J. Balog, unpublished data). Table 5 shows that the relaxed line had significantly higher uric acid, when compared with the other two lines. Finally, the relaxed line had significantly higher serum triglycerides, when compared with the resistant and the susceptible lines (Table 5). Whether or not selection for ascites resistance or susceptibility affected lipid metabolism in these lines is unclear from this one trial.

In conclusion, the results of this trial at local altitude confirm that the lines selected from hypobaric data respond to cold stress in a manner that typifies their means of selection. When cold temperature is used as an inducing stressor, the hypobarically selected, ascites-resistant birds are still resistant and the hypobarically susceptible birds are still susceptible.

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REFERENCES

Acar, N., F. G. Sizemore, G. R. Leach, R. F. Wideman, Jr., R. L. Owen, and G. F. Barbato. 1995. Growth of broiler chickens in response to feed restriction regimes to reduce ascites. Poult. Sci. 74:833–843.

Arce, J., M. Berger, C. L. Coello. 1992. Control of ascites syndrome by feed restriction techniques. J. Appl. Poult. Res. 1:1–5.

Anthony, N. B., J. M. Balog, J. D. Hughes, L. Stamps, M. A. Cooper, B. D. Kidd, X. Liu, G. R. Huff, W. E. Huff, and N. C. Rath. 2001. Genetic selection of broiler lines that differ in their ascites susceptibility 1. Selection under hypobaric conditions. Pages 327–328 in Proceedings of the 13th European Symposium on Poultry Nutrition, Blankenberge, Belgium. World Poultry Science Association, Belgium.

Balog, J. M., N. B. Anthony, M. A. Cooper, B. D. Kidd, G. R. Huff, W. E. Huff, and N. C. Rath. 2000a. Ascites syndrome and related pathologies in feed restricted broilers raised in a hypobaric chamber. Poult. Sci. 79:318–323.

Balog, J. M., G. R. Huff, N. C. Rath, and W. E. Huff. 2000b. Effect of dietary aspirin on ascites in broilers raised in a hypobaric chamber. Poult. Sci. 79:1101–1105.

Burton, R. R., E. L. Besch, and A. H. Smith. 1968. Effect of chronic hypoxia on pulmonary arterial blood pressure of the chicken. Am. J. Physiol. 14:1438–1442.

Cooper, M. A., J. M. Balog, K. Halterman, B. Kidd, L. Mullikin, and N. B. Anthony. 1998. Effect of feed restriction in broilers raised at simulated high altitude 1. Ascites incidence and weight gain. Poult. Sci. 77(Suppl. 1):82. (Abstr.)

Cueva, S., H. Sillau, A. Valenzuela, and H. Ploog. 1974. High altitude induced pulmonary hypertension and right heart failure in broiler chickens. Res. Vet. Sci. 16:370–374.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1–42.

Hall, S. A., and N. Machicao. 1968. Myocarditis in broiler chickens reared at high altitude. Avian Dis. 12:75–84.

Hernandez, A. 1987. Hypoxic ascites in broilers: A review of several studies done in Colombia. Avian Dis. 31:171–183.

Hughes, J. D., N. B. Anthony, J. M. Balog, G. R. Bayyari, and W. E. Huff. 1995. High altitude simulation as a means for inducing ascites in broilers. Poult. Sci. 75(Suppl.1):6. (Abstr.)

Huchzermeyer, F. W., and A. M .C. DeRuyck. 1986. Pulmonary hypertension syndrome associated with ascites in broilers. Vet. Rec. 119:94.

Huchzermeyer, F. W., A. M. C. DeRuyck, and H. Van Ark. 1988. Broiler pulmonary hypertension syndrome. III. Commercial broiler strains differ in their susceptibility. Onderstepoort J. Vet. Res. 55:5–9.

Julian, R. J. 1993. Ascites in poultry (review article). Avian Pathol. 22:419–454.

Lubritz, D. L., J. L. Smith, and B. N. McPherson. 1995. Heritability of ascites and the ratio of right to total heart weight in broiler breeder male lines. Poult. Sci. 74:1237–1241.

Maxwell, M. H., G. W. Robertson, and S. Spence. 1986. Studies on an ascitic syndrome in young broilers 1. Haematology and pathology. Avian Pathol. 15:511–524.

Maxwell, M. H., S. G. Tullet, and F. G. Burton. 1987. Haematology and morphological changes in young broiler chicks with experimentally induced hypoxia. Res. Vet. Sci. 43:331–338.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Olander, H. J., R. R. Burton, and H. E. Adler. 1967. The pathophysiology of chronic hypoxia in chickens. Avian Dis. 11:609–620.

Pavlidis, H. O., L. Stamps, J. D. Hughes, Jr., M. A. Cooper, J. M. Balog, W. E. Huff, G. R. Huff, N. C. Rath, and N. B. Anthony. 2002. Divergent selection for ascites incidence in chickens. Poult. Sci. 81(Suppl. 1):35. (Abstr.)

Peacock, A. J., C. K. Pickett, K. M. Morris, and J. T. Reeves. 1988. Spontaneous pulmonary hypertension in rapidly growing broiler chickens reared at sea level. Am. Rev. Respir. Dis. 137:106. (Abstr).

Riddell, C. 1991. Developmental, metabolic, and miscellaneous disorders. Pages 839–841 in Diseases of Poultry. 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr., ed. Iowa State University Press, Ames, IA.

Robinson, F. E., H. L. Classen, J. A. Hanson, and D. K. Onderka. 1992. Growth performance, feed efficiency, and the incidence of skeletal and metabolic disease in full-fed and feed restricted broiler and roaster chickens. J. Appl. Poult. Res. 1:33–41.

SAS Institute. 1988. SAS/STAT User's Guide. 1988 Edition. SAS Institute Inc., Cary, NC.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.

Wideman, R. F., Jr., Y. K. Kirby, W. G. Bottje, R. W. Moore, and R. C. Vardeman. 1995. Furosemide reduces the incidence of pulmonary hypertension syndrome (ascites) in broilers exposed to cool temperatures. Poult. Sci. 74:314–322.

Wideman, R. F., Jr., T. Wing, Y. K. Kirby, M. F. Forman, N. Marson, C. D. Tackett, and C. A. Ruiz-Feria. 1998. Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. Poult. Sci. 77:1565–1573.

Yersin, A. G., W. E. Huff, L. F. Kubena, M. A. Elissalde, R. B. Harvey, D. A. Witzel, and L. E. Giroir. 1992. Changes in hematological, blood gas, and serum biochemical variables in broilers during exposure to simulated high altitude. Avian Dis. 36:189–197.

Yu, M. W., and F. E. Robinson. 1992. The application of short-term feed restriction to broiler chicken production: A review. J. Appl. Poult. Res. 1:147–153.